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Pseudopyricularia cyperi, a new record for Iran

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Abstract—During a survey of sedge plants in northern Iran, three specimens of *Pseudopyricularia cyperi* were isolated from *Cyperus* sp. Their taxonomical identity was established by their spore and conidiophore morphology and ITS rDNA sequence analysis. The specimens are described and illustrated. *Pseudopyricularia cyperi* is a new record for Iran.

Key words—*Magnaporthales,* phylogeny, *Pyriculariaceae,* taxonomy

Introduction

Pyriculariaceae was established as a new family of the *Magnaporthales* by Klaubauf & al. (2014). The family contains ten genera: *Bambusicularia, Barretomyces, Deightoniella, Macgarvieomyces, Neocordana, Neopyricularia, Proxipyricularia, Pseudopyricularia, Pyricularia,* and *Xenopyricularia* (Klaubauf & al. 2014, Hernández-Restrepo & al. 2015). Some species originally described in *Pyricularia* have been transferred to these new genera; e.g., *Macgarvieomyces borealis, Proxipyricularia zingiberis, Neopyricularia commelinicola, Pseudopyricularia higginsii,* and *Xenopyricularia zizaniicola.*

Pyricularia higginsii Luttr. was transferred to *Dactylaria* by Ellis (1976), but Bussaban & al. (2003) maintained the species in *Pyricularia* based on rDNA ITS sequence analysis. On the basis of analyses of five gene regions, Klaubauf

& al. (2014) placed some isolates previously identified as *Pyricularia higginsii* in a new genus *Pseudopyricularia* and separated them into *Pseudopyricularia higginsii* and two new species, *P. cyperi* and *P. kyllingae*.

Additional species since described or combined in *Pseudopyricularia* include *P. bothriochloae, P. hagahagae, P. hyrcaniana, P. iraniana,* and *P. persiana* (Crous & al. 2015, 2018; Pordel & al. 2017; Marin-Felix & al. 2017; Jayawardena & al. 2019). *Pseudopyricularia* species are primarily distinguished from *Pyricularia* sensu stricto by having short, determinate, brown conidiophores with an apical rachis with flat-tipped denticles; they are similar to each other in conidial size and can be resolved only by phylogenetic analysis (Klaubauf & al. 2014; Pordel & al. 2015, 2017). Pordel & al. (2017) reassessed the morphological criteria, and re-described *Pseudopyricularia* conidia as obclavate, fusiform, cylindrical, and 1–2-septate.

Here we describe and illustrate *Pseudopyricularia cyperi* from Iranian specimens and compare them with other species in *Pseudopyricularia*.

Materials & methods

Sampling and fungal isolates

Plant material was obtained from Mazandaran Province in Iran during the summer and fall of 2012 and 2015. Leaves of *Cyperus* sp. with leaf spot symptoms were collected and stored in a refrigerator at 4 °C until used. To induce sporulation of *Pseudopyricularia*, leaf pieces were surface sterilized for 2 min in 1% sodium hypochlorite, dried on filter paper, and then incubated on wet filter paper at 25 °C. Conidia produced on these surface-sterilized leaf pieces were transferred to water agar (WA). Single hyphal tips emerging from germinating conidia were then transferred to potato dextrose agar (PDA) medium (Pordel & al. 2015).

Living cultures have been deposited in the herbarium in the Department of Plant Protection, Faculty of Agricultural Sciences & Engineering, University of Tehran, Karaj, Iran (UTFC).

Morphological characterization

Cultures were grown on oatmeal agar (OA) and PDA to determine overall colony morphology and characteristics. Micromorphological characters were determined from colonies grown on synthetic nutrient-poor agar (SNA; Nirenberg 1976), and WA supplemented with pieces of rice leaves. Plates were incubated at 23–25 °C under a regime of 12 h dark/12 h near-ultraviolet light and examined for sporulation after 1–3 wks. Measurements and microphotographs were taken from slide mounts in lactophenol and lactophenol cotton blue. Measurements were taken from 70–100 conidiophores and 200 conidia. Photographs were taken using a Sony digital camera mounted on an Olympus BH2 microscope.

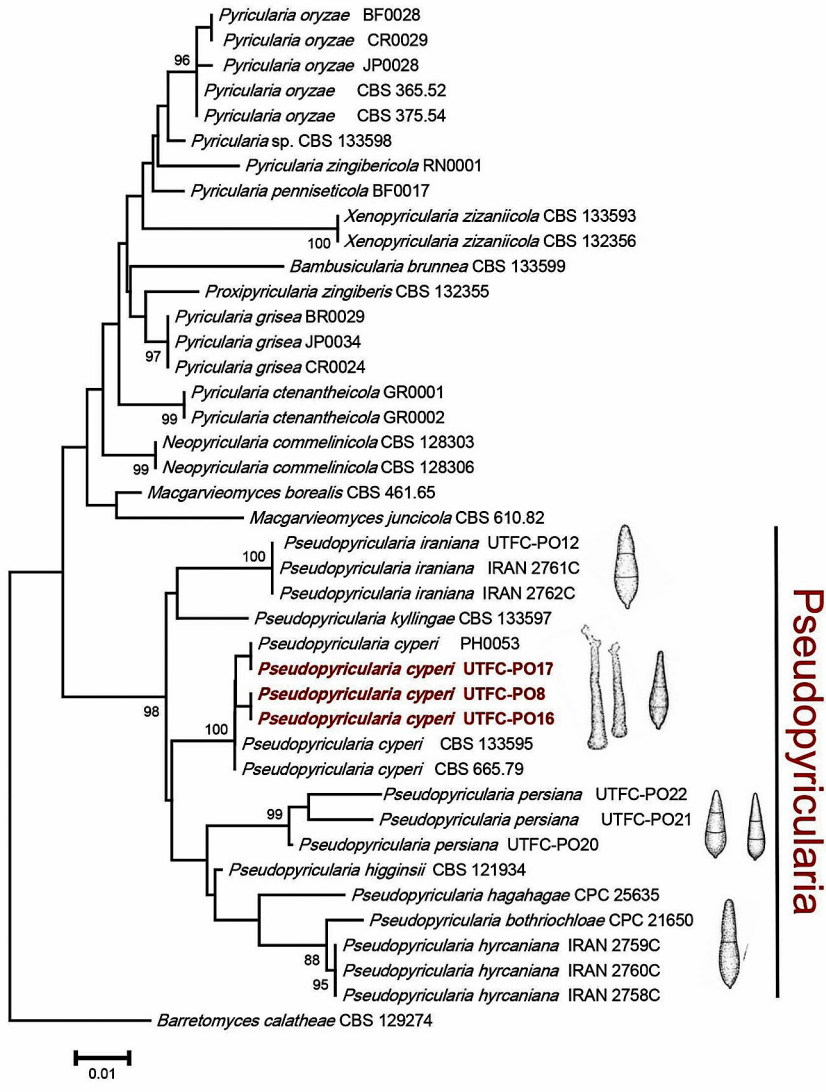

Fig. 1. Maximum likelihood tree inferred from ITS sequence of *Pyriculariaceae* species. Branch values of ML >80% are noted above internodes. Strains of *Pseudopyricularia cyperi* from Iran are in bold.

Phylogenetic analysis

The three isolates of *Pseudopyricularia cyperi* collected in Iran were used for phylogenetic analysis. Total genomic DNA was extracted using the protocol of Zhong & Steffenson (2001) and the ITS region was amplified according to Klaubauf & al. (2014). Additional ITS sequences were downloaded from GenBank. A total of 41 nucleotide sequences (including GenBank sequences originating from Klaubauf & al. 2014) were analyzed. *Barretomyces calatheae* was used as outgroup (Klaubauf & al. 2014).

The sequences were edited by CLC Genomic Workbench 10.1 and aligned using MUSCLE (Edgar 2004). Phylogenetic analyses were performed with MEGA v. 6 (Tamura & al. 2013). A phylogenetic tree was constructed using the maximum likelihood method based on the Kimura two-parameter model (Kimura 1980). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. All positions with less than 95% site coverage were eliminated, permitting fewer than 5% alignment gaps, missing data, and ambiguous bases at any position. The final ITS dataset comprised 296 positions.

Results

Phylogenetic analysis

Our phylogenetic analyses grouped *Pseudopyricularia* species together in one clade within *Pyriculariaceae* with a high (bootstrap >98%) statistical support (Fig. 1). These species have variously shaped 1–2-septate conidia. All are pathogenic to plants but have different hosts and have likely evolved separately. The phylogeny (Fig. 1) demonstrates a 100% MLBP support for a close relationship between known *P. cyperi* isolates and the strains isolated from *Cyperus* sp. in Iran, supporting classification of the Iranian samples as *Pseudopyricularia cyperi*. In particular, isolate UTFC-P016 has the same ITS sequences as *P. cyperi* CBS 133595 (ex-holotype; from Japan) and isolate UTFC-P017 has the same ITS sequences as *P. cyperi* PH0053 (ex-paratype; from Philippines).

Taxonomy

Pseudopyricularia cyperi Klaubauf, M.-H. Lebrun & Crous, Stud. Mycol. 79: 110. 2014. Figs 2, 3

Colonies grown on PDA after 1 wk at 23–25 °C white, reaching 40 mm diam. Conidiophores solitary, erect, straight or curved to geniculate, branched,

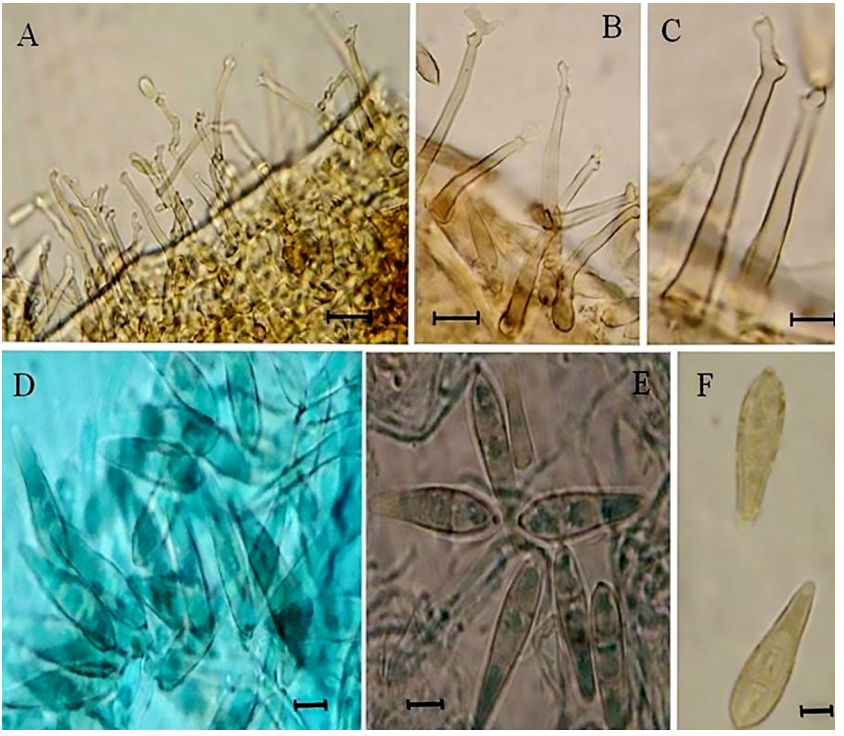

Fig. 2. *Pseudopyricularia cyperi* (UTFC-PO8). A–C. Solitary, erect, branched, and unbranched conidiophores; D–F. Conidia. Scale bars = 10 µm.

medium brown, smooth, 40–100 × 3–4 µm. Conidiogenous cells integrated, terminal and intercalary, pale brown, smooth, forming a rachis with several protruding, flat-tipped denticles. Conidia solitary, obclavate, medium brown, smooth to finely roughened, 2-septate, 22–29 × 5–6 µm; hilum truncate, slightly protruding, unthickened, not darkened.

Specimens examined—On infected leaves of *Cyperus* sp. **IRAN**, **Mazandaran province**, Amol, 25 July 2012, Adel Pordel cy2k (UTFC-PO8; GenBank KP144446), Adel Pordel cy4-1 (UTFC-PO16; GenBank MF768983), Adel Pordel cy2k1 (UTFC-O17 GenBank MF768984).

Comments—The morphology of the Iranian specimens cited above agree with the protologue description of *Pseudopyricularia cyperi* (Klaubauf & al. 2014). This species is morphologically similar to *P. higginsii* (Luttr.)

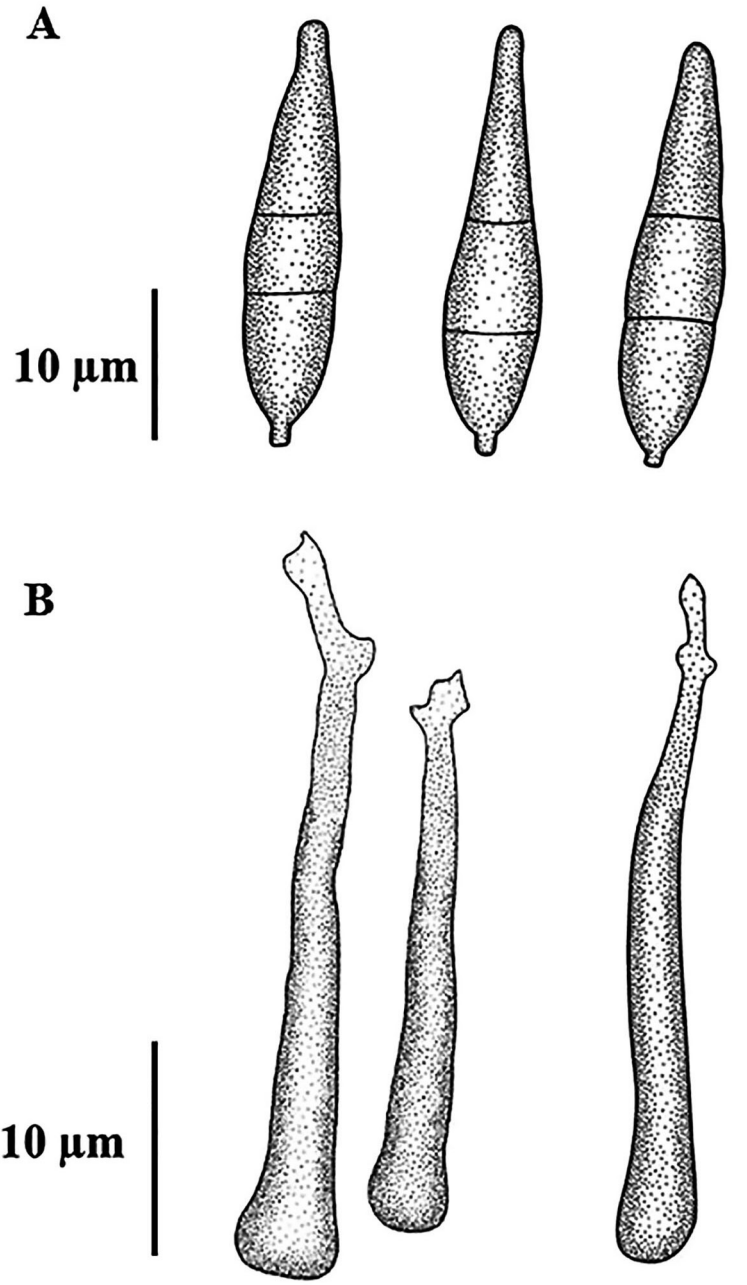

Fig. 3. *Pseudopyricularia cyperi* (UTFC-PO8). A. Conidia; B. Conidiophores

Klaubauf & al., which differs by its longer and slightly wider conidia (17.5–36.5 × 5.3–6.5 µm, Luttrell 1955; 20–36 × 5–6 µm, Ellis 1976). This represents the first report of *Pseudopyricularia cyperi* in Iran.

Discussion

The order *Magnaporthales* has been reclassified into three families, *Magnaporthaceae*, *Pyriculariaceae*, and *Ophioceraceae* (Klaubauf & al. 2014, Luo & al. 2015, Pordel & al. 2017). The *Pyriculariaceae* is characterized by *Pyricularia*-like asexual states and pathogenicity to *Poaceae* or other monocotylenous plants. Phylogenetic analysis based on five gene regions allowed definition of several new genera and species in *Pyriculariaceae* (Klaubauf & al. 2014).

Previously, several species of *Pseudopyricularia* were unrecognized and placed in the *P. higginsii* complex. Based on phylogenetic analysis, new *Pseudopyricularia* species were identified in this clade (Klaubauf & al. 2014, Pordel & al. 2017). *Pseudopyricularia* and *Macgarvieomyces* are morphologically and phylogenetically similar (Klaubauf & al. 2014, Pordel & al. 2017). *Macgarvieomyces* is distinguished from *Pseudopyricularia* and *Pyricularia* sensu stricto by its production of chlamydospores, mostly unbranched conidiophores, and narrowly obclavate, granular, guttulate and 1-septate conidia (Pordel & al. 2017). *Pseudopyricularia bothriochloae* (Crous & Cheew.) Y. Marín & Crous is closely related to *P. hyrcaniana* Pordel & Jav.-Nikkh (both with 1-septate conidia), while *P. hagahagae* Crous & M.J. Wingf. (with 2-septate conidia) is clearly separated from them phylogenetically (Fig. 1). The phylogeny also supports five species (*P. higginsii*, *P. iraniana* Pordel & Jav.-Nikkh, *P. cyperi*, *P. persiana* G. Ghorbani & al., and *P. kyllingae* Klaubauf & al.) as separate from species having 1-septate conidia. *Pseudopyricularia cyperi* strains from Iran, which lie in *P. cyperi* clade, are morphologically similar to all previous records of this species from *Cyperus* sp. in other countries (Klaubauf & al. 2014; Borromeo & al. 1993, as "*P. higginsii*").

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